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## **Introduction: History and Future of Ranaviruses**

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# Introduction: History and Future of Ranaviruses

Matthew J. Gray and V. Gregory Chinchar

*What a wonderful bird the frog are—  
When he stand he sit almost;  
When he hop, he fly almost.  
He ain't got no sense hardly;  
He ain't got no tail hardly either.  
When he sit, he sit on what he ain't got almost.*

Anonymous

Allan Granoff (1923–2012) serendipitously isolated the first ranaviruses (Granoff et al. 1966) while attempting to generate cell lines that would support the replication of Lucke herpesvirus. Although one of Allan's isolates, Frog virus 3 (FV3), subsequently became the best-characterized member of both the genus (*Ranavirus*) and the family (*Iridoviridae*); the impact of that discovery was not fully appreciated at the time. FV3 was neither the first iridovirus to be recognized as a pathogen of lower vertebrates or the first isolated. Those honors belonged to lymphocystis disease virus (LCDV) and invertebrate iridovirus 1 (IIV1), respectively (Wissenberg 1965; Xeros 1954). LCDV is responsible for a generally non-life threatening, but disfiguring, disease in fish characterized by the appearance of wart-like growths on the skin and (rarely) internal organs, whereas IIV1 is the causative agent of latent and patent infections in crane fly larvae. Despite its lack of primacy, FV3 was studied because, in keeping with the mission of St. Jude Hospital, it was initially thought to be linked to adenocarcinoma in frogs and thus could be a useful model of human malignancies. Furthermore, unlike LCDV and IIV1, it could be readily grown in cultured cells and was thus amenable to detailed molecular characterization. Although its role in tumor development was soon proven incorrect, FV3 served as a gateway into understanding the replication strategy of a heretofore poorly studied virus family. Moreover, over the next 20 years, its study led to

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important insights not only into iridovirus replication, but also eukaryotic biology, virus evolution, and host–virus interactions.

Elucidating the molecular and cellular events of FV3 replication occupied Allan, his co-workers, and others in the USA and Europe from the discovery of FV3 in 1965 until the early 1990s (Murti et al. 1985; Williams 1996). However, despite the molecular insights gained in these studies, investigations of FV3 and other members of the family languished for a variety of reasons. After some initial optimism, it was clear that invertebrate iridoviruses were not suitable, as was baculovirus, as an insect biocontrol agent. Furthermore, FV3 and related vertebrate iridoviruses were initially viewed as minor pathogens, because few outbreaks of ranaviral disease were reported, and those that were, appeared to have minor effects on populations. In addition, unlike LCDV, there was little evidence of infection among ecologically or commercially important fish species. Therefore, even before the recent emphasis on “translational research,” iridovirus studies took a backseat to more medically and commercially relevant poxviruses and herpesviruses.

However, beginning in the mid-1980s and continuing to the present, this sanguine view of ranaviruses slowly changed, as an increasingly large number of isolates similar to, but not necessarily identical with, FV3 were linked to die-offs of fish, reptiles, and amphibians (frogs, toads, and salamanders) of both ecological and commercial importance (Chinchar et al. 2009). To date, cases of ranavirus infection and disease have been documented on six continents and in at least 175 species of ectothermic vertebrates (Duffus et al. 2015). It is unclear whether the global emergence of ranaviruses is a reflection of their increased virulence or dissemination (via natural or human-related activities) or increased surveillance coupled with better diagnostic and detection mechanisms. Regardless of the reasons, ranaviruses are now viewed as pathogenic agents capable of infecting all classes of ectothermic vertebrates (fish, reptiles, and amphibians) and, depending upon the specific virus, host, and environmental factors, triggering significant morbidity and mortality.

The family *Iridoviridae* currently contains five genera, two of which infect invertebrates (*Iridovirus* and *Chloriridovirus*) and three that infect only ectothermic vertebrates (*Lymphocystivirus*, *Megalocytivirus*, and *Ranavirus*; Jancovich et al. 2015a). Lymphocystiviruses and megalocytiviruses only infect fish, whereas, as indicated above, ranaviruses target fish, amphibians, and reptiles. Infection of “higher” vertebrates (i.e., birds and mammals) has not been reported. However, this block likely reflects a temperature limit above which the virus cannot replicate (approximately 32 °C), and not a lack of suitable cellular receptors, as ranaviruses can replicate in mammalian cell lines (e.g., baby hamster kidney) when incubated at 30 °C. Ranaviruses also cause apoptotic cell death in mammals even if the pathogen has been inactivated by heat or radiation (Grayfer et al. 2015). Thus, ranaviruses represent a group of pathogens that possesses a wide host range and the potential to affect diverse populations of vertebrate species around the globe.

The question frequently arises, “Are ranaviruses a significant threat to wildlife?” We believe the answer is, “Yes,” but that the seriousness of the threat is dependent

upon a number of factors. Brunner et al. (2015) describe how ranaviruses could contribute to species declines using epidemiological theory and results from mathematical simulations. However, because there have been very few long-term longitudinal studies on populations with reoccurring ranavirus die-offs, data necessary to address population and species declines are scarce. Recent studies are beginning to address this deficiency. Stephen Price and colleagues recently reported ranavirus-induced declines in three amphibian species at several sites in northern Spain (Price et al. 2014). Amber Teacher and colleagues analyzed an 11-year dataset in England, and found about an 80 % decline in common frog abundance at ranavirus die-off sites (Teacher et al. 2010). Jim Petranka and several other ecologists have observed no recruitment in consecutive years at sites with ranavirus die-offs (Petranka et al. 2003; Wheelwright et al. 2014). Julia Earl showed in closed populations of wood frogs that reoccurring outbreaks of ranavirus could result in population extinction in as quickly as 5 years (Earl and Gray 2014). These studies suggest that several elements are in place (e.g., high susceptibility among several host species, possible density-independent transmission) for ranaviruses to cause local population extinction and thereby contribute to species declines. However, to date, species extinction due to ranaviral disease has not been reported. This uncertainty emphasizes the need for more intensive investigations in ranavirus surveillance and population monitoring, which is outlined in Gray et al. (2015). Importantly, we should not sit idly until there is definitive evidence of species extinctions due to ranavirus. The writing is on the wall suggesting its potential threat, especially considering that many rare species are hosts for ranaviruses. For example, the highly endangered Chinese giant salamander (*Andrias davidianus*, Geng et al. 2010), gopher tortoise (*Gopherus polyphemus*, Westhouse et al. 1996), dusky gopher frog (*Lithobates sevosus*, Sutton et al. 2014), and boreal toad (*Anaxyrus boreas boreas*, J. Chaney, M. Gray, and D. Miller, University of Tennessee, unpublished data) are very susceptible to ranaviral disease. Additional investigations are needed to identify other rare species that are highly susceptible (Gray et al. 2015). In captivity, 100 % mortality of hosts is commonly observed likely due to abundant hosts, guaranteed transmission, and stress associated with these environments (Waltzek et al. 2014). Several species of economic (e.g., bullfrogs, Mazzoni et al. 2009; grouper, Qin et al. 2001) and conservation concern (e.g., pallid sturgeon, Waltzek et al. 2014; Chinese giant salamander, Geng et al. 2010; Cunningham et al. 2015) have experienced catastrophic losses in captivity due to ranaviruses. Given this preliminary information on the possible effects of ranaviruses on highly susceptible hosts, we believe it is reasonable to consider this pathogen a serious threat to the biodiversity of ectothermic vertebrate species.

Another question that frequently arises is, “Are ranaviruses emerging?” In other words, “Are ranaviruses increasing in distribution, prevalence, or host range?” Again, this is a challenging question to answer, but there is information that suggests, “Yes.” Andrew Storfer provided evidence, based on a lack of coevolutionary history between virus and host, that *Ambystoma tigrinum virus* (ATV) emerged in some locations (Storfer et al. 2007). His work suggests that emergence of ATV was likely a consequence of the trade in larval salamanders as fishing bait and

the anthropogenic translocation of sublethally infected salamanders over large geographic distances (Storfer et al. 2007; Picco and Collins 2008). Thomas Waltzek at the University of Florida is currently sequencing the entire genomes of dozens of ranaviruses from around the globe, which will enable him to look at phylogeographic patterns, and identify areas of recent introductions. In general, pathogens emerge in populations either because they are novel or due to an increase in environmental stressors that decrease host immune function. As described above, there is support for the first hypothesis, and it is likely a consequence of pathogen pollution (i.e., the human movement of infected animals or contaminated fomites over large geographic distances, Cunningham et al. 2003). Furthermore, although research is limited, there is evidence that insecticides, herbicides, and the use of wetlands by cattle can act as stressors and increase the chance of ranavirus emergence (Forson and Storfer 2006; Gray et al. 2007; Kerby et al. 2011). In the past 4 years, >90 % of the cases of ranavirus infection and disease have been reported (Duffus et al. 2015). While enhanced awareness of ranaviruses and increased sampling efforts probably contributes to increased detections, it is unlikely that these factors are solely responsible.

In this contribution, we provide a comprehensive and current review of ranavirus taxonomy (Jancovich et al. 2015a), virus distribution (Duffus et al. 2015), host-pathogen ecology and evolution (Brunner et al. 2015), viral replication strategies (Jancovich et al. 2015b), host antiviral immunity and viral countermeasures (Grayfer et al. 2015), ranavirus pathology and diagnosis (Miller et al. 2015), and suggestions for the design and analysis of ranavirus studies (Gray et al. 2015). Collectively, this work provides an up-to-date overview of ranaviruses and their impacts on host organisms, and reflects the contributions of investigators (i.e., molecular virologists, immunologists, ecologists, veterinary pathologists, population biologists) possessing diverse skills, but united in their interest in ranaviruses and the diseases they cause.

In addition to this book, professionals around the globe have been working together to learn about ranaviruses. For example, two international symposia devoted to ranaviruses (Minneapolis, Minnesota, 2011; Knoxville, Tennessee, 2013) brought together scientists interested in understanding ranaviruses and their disease potential (Lesbarrères et al. 2012; Duffus et al. 2014). A third symposium is planned for Gainesville, Florida, USA in 2015. Between the 2011 and 2013 symposia, investigators interested in ranaviruses founded the Global Ranavirus Consortium (GRC). The goal of the GRC is to facilitate communication and collaboration among scientists and veterinarians conducting research on ranaviruses and diagnosing cases of ranaviral disease. Specifically, the GRC aims to: (1) advance knowledge in all areas of ranavirus biology and disease, (2) facilitate multidisciplinary, scientific collaborations, (3) disseminate information on ranaviruses, and (4) provide expert guidance and training opportunities. The GRC accomplishes these goals by hosting a biennial symposium, organizing regional workshops and discussion groups, and maintaining a website (<http://ranavirus.org>) with various resources including a current list of ranavirus publications and laboratories that test for the pathogen. They also are leading an effort to create a Global Ranavirus Reporting System, which will be an online data management system that allows cases of ranavirus infection and disease

to be uploaded, mapped, and downloaded by users. The GRC announced charter membership in 2015.

So, what does the future look like for ranaviruses? We are just beginning to scratch the surface in understanding the complex interactions between these pathogens and their diverse hosts. More information is needed on basic molecular biology of ranaviruses, immunological responses of hosts, and resulting pathologies as outlined in Jancovich et al. (2015b), Grayfer et al. (2015), and Miller et al. (2015). These data are fundamental to understanding underlying mechanisms to ranavirus–host interactions. More research is needed to understand why ranaviruses emerge in certain areas. Are the factors related to basic epidemiological principles (e.g., density-independent transmission), natural stressors (e.g., breeding), anthropogenic stressors (e.g., pesticides), or recent pathogen introduction? To answer these questions laboratory experiments need to be coupled with field research, and host health assessments performed by immunologists and pathologists. We also do not understand the potential effects of climate change on ranavirus distribution and pathogenicity. Given that many ranaviruses replicate faster at warmer temperatures (Ariel et al. 2009), it is possible that atmospheric warming could contribute to emergence. For amphibians, we also know that rapidly drying breeding sites, which could increase in some regions due to climate change, stress larvae and may contribute to disease severity. Another factor is the apparent increase in pathogenicity of ranaviruses associated with die-offs in captive facilities, typically those associated with aquaculture or frog farms (Brunner et al. 2015). If this hypothesis is correct, trade of ectothermic vertebrates could be moving highly virulent ranavirus strains around the globe, which emphasizes the need to implement regulations on pretesting animals as recommended by the World Organization for Animal Health (Schloegel et al. 2010). When we think about commerce, international trade is generally of greatest concern. However, as Andrew Storfer’s study showed, movement over small geographic distances (several 100 km) can be enough to result in emergence (Storfer et al. 2007). Interestingly, we recently finished controlled experiments that suggest as little as 100 km may be far enough to result in differences in coevolutionary history between ranavirus and a host, resulting in increased levels of mortality (P. Reilly, M. Gray, D. Miller, University of Tennessee, unpublished data). Collectively, the data suggest that ranavirus emergence likely reflects the combined effects of human-induced spread, increased environmental stress, depressed host immunity, and enhanced virulence.

In the nearly 50 years since the discovery of FV3, ranaviruses have gone from being merely a curiosity (i.e., a virus family with interesting molecular aspects but of little commercial or medical importance) to a genus whose members have profound impacts, both potentially and actually, on animal health and well-being. Moreover, studies of amphibian responses to ranavirus infection have advanced our understanding of antiviral immunity in lower vertebrates and suggested pathways for vaccine development. This work has validated the view proposed more than 30 years ago that unusual organisms are studied, not just because they are odd, but also because they provide insights into fundamental biological processes common to all organisms.

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